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# Relationship between primary producers and bacteria in an oligotrophic sea—the Mediterranean and biogeochemical implications

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ABSTRACT: The proverbial blue colour of the Mediterranean reflects some of the most extreme oligotrophic waters in the world. Sea-surface Sea-viewing Wide Field-of-view Sensor (SeaWiFS) satellite data show the relatively clear, pigment poor, surface waters of the Mediterranean with a generally increasing oligotrophy eastward, apparent even from space. Integrated over depth, however, the east and west Mediterranean show similar amounts of phytoplankton and bacterial biomass. By contrast, primary production and bacterial production are 2 to 3 times lower in the eastern Mediterranean than in the west. However, the relationship between bacterial production and primary production in the east and west are significantly different. While bacterial production is directly proportional to primary production in the east, in the west it increases as approximately the square root of primary production. This suggests that the bacteria in the west are relatively decoupled from local contemporaneous primary production. In contrast, the gradient of close to 1 in the log bacterial production versus log primary production relationship in the east suggests less temporal decoupling and, therefore, less seasonal accumulation of DOC. In addition, the constant proportionality between bacterial and primary production of 0.22, which, if all primary products are respired, gives an estimated geometric mean bacteria growth efficiency of 22% (95% confidence limits of 17 and 29%) for data in the eastern Mediterranean. Our data suggest that the degree of bacteria-phytoplankton coupling has an important effect on apparent trends between bacterial and phytoplankton production in high frequency data. The combination of low primary production and bacterial dominance of secondary production in the east is also of significance as it could account for the low fisheries production, the low vertical flux of material and low biomass of benthic organisms in the region.

KEY WORDS: Bacteria · Phytoplankton · Bacterial growth efficiency · Ocean productivity · Oligotrophy · Mediterranean

## INTRODUCTION

The Mediterranean Sea has high evaporation rates and low land run-off, resulting in a deficit in its hydrological balance. Nutrient-depleted Atlantic water flows into the Mediterranean through the narrow (ca 4 $km^2$) Strait of Gibraltar (Béthoux et al. 1992) and, after circulating the basin, exits the same way with nearly 10% more salt content (Milliman et al. 1992). There is increasing nutrient depletion from west to east, with a particularly pronounced gradient for phosphorus (Krom et al. 1991). The basin-wide cyclonic circulation of nutrient-depleted water (Dugdale & Wilkerson 1988), hot, dry and seasonal climate and low land run-off contribute to the low productivity of the sea and the west-east trend in oligotrophy (Fig. 1, Table 1). The aim of this paper is to investigate the coupling between bac-

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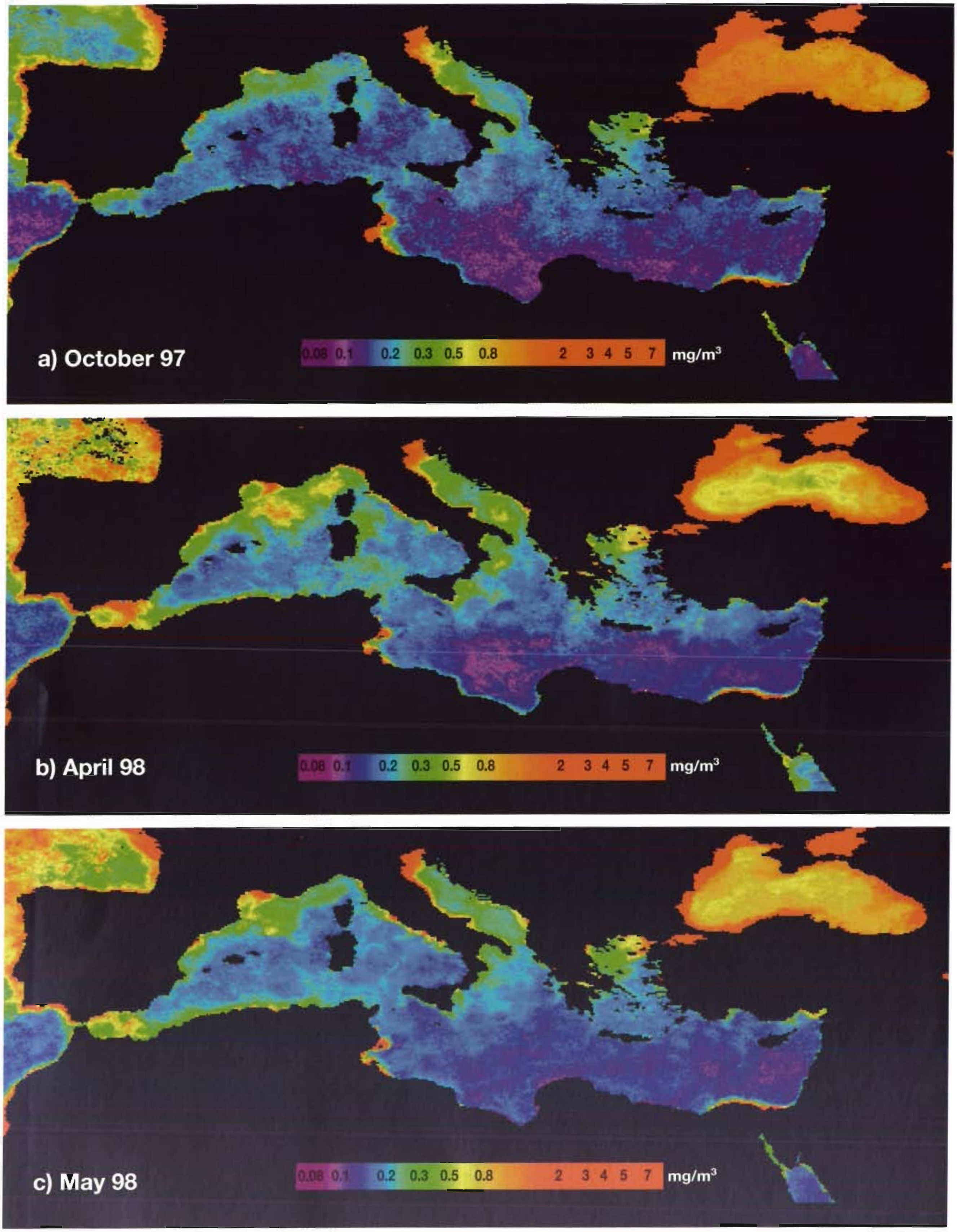
a) October 97
0.08 0.1 0.2 0.3 0.5 0.8 2 3 4 5 7 mg/m³
b) April 98
0.08 0.1 0.2 0.3 0.5 0.8 2 3 4 5 7 mg/m³
c) May 98
0.08 0.1 0.2 0.3 0.5 0.8 2 3 4 5 7 mg/m³

Fig. 1. Ocean colour Sea-viewing Wide Field-of-view Sensor (SeaWiFS) from Orbital Sciences Coorporations' SeaStar satellite estimates of chlorophyll *a* concentrations (mg $m^{-3}$) in a monthly composite during (a) October 1997, (b) April 1998 and (c) May 1998

terial and primary production in the western and eastern Mediterranean and evaluate its significance to the west-east trend in productivity.

## METHODS

Basin-wide and regional sea-surface chlorophyll *a* concentrations were estimated using a Sea-viewing Wide Field-of-view Sensor (SeaWiFS) Version 2 chlorophyll product from NASA (Fig. 1) in order to examine seasonal sea surface concentrations in the western and eastern Mediterranean basins (Table 1).

Chlorophyll was determined from water samples using a fluorometric method (Yentch & Menzel 1963, Holm-Hansen & Riemann 1978). Primary production was measured using the $NaH^{14}CO_3$ method (Steeman-Nielsen 1953, Dandonneau & Le Bouteiller 1992) and the phytoplankton efficiency (PE) estimated by primary production/chlorophyll.

Bacteria were enumerated directly by epifluorescent microscopy and staining with the DAPI fluorochrome (Porter & Ferg 1980) on freshly preserved, filtered (Turley & Hughes 1992) and sonicated samples (Turley et al. 1996). Bacterial biomass was calculated from cell numbers using the conversion factor 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987).

Bacterial production was calculated using the theoretical approach of Kirchman (1993) by measuring leucine incorporation into bacterial protein after the addition of 10 nM $^3$H-leucine (Chin-Leo & Kirchman 1988).

The above analyses were carried out at the sites in the western and eastern Mediterranean basins shown in Fig. 2.

*T*-tests were carried out on logged data, where appropriate, to test for significant differences for western and eastern integrated data used in Table 2. Regression analysis (Table 3) was applied to the western and eastern bacterial and primary production data in Fig. 3 to test for a significant difference between the 2 regressions of the data from the 2 regions.

## RESULTS AND DISCUSSION

Both nitrogen and phosphorous can be limiting nutrients for phytoplankton and bacterial growth in the Mediterranean during summer (Dugdale & Wilkerson 1988, Béthoux et al. 1992, Berland et al. 1990, Krom et al. 1991, Estrada et al. 1993, Thingstad & Rassoulzadegan 1995). Despite the deep chlorophyll maximum (DCM) characteristic of the Mediterranean (Estrada et al. 1993, Lefèvre et al. 1997), there is a west-east trend in surface chlorophyll *a* concentration seen from space during spring and summer (Fig. 1, Table 1). This is even more apparent if the anthropogenically enriched waters of the Adriatic are excluded from the eastern basin (Table 1). The DCM is, in general, over 30 m deeper in the east but, in contrast to the SeaWiFS images of surface chlorophyll, the integrated chlorophyll is similar in the west and east (Table 2). The integrated primary production in the west, however,

Table 1. Comparison of sea surface chlorophyll *a* (chl *a*) concentrations between the western and eastern Mediterranean. The regional sea-surface chl *a* data were extracted from the SeaWiFS images shown in Fig. 1 using hand drawn masks. W Med. is the geometric mean of all data west of a line between Sicily and Africa. E Med is the geometric mean of all data east of the line. E Med (no Adriatic) is the E Med excluding the Adriatic (area north of a line between the closest point of the heel of Italy and the Balkans). The raw scaled data were converted to logchl *a* and statistics calculated for data points greater than zero (logchl *a* > 0.01). SD (sqrt variance) is in units of logchl *a*. Data used is from SeaWiFS Version 2 chlorophyll product from NASA

| | Image | Mean units mg chl *a* $m^{-3}$ | SD in units of logchl *a* | W:E | W:E (no Adriatic) |
|---|---|---|---|---|---|
| **October 1997** | W Med | 0.170 | 0.12 | 1.07 | 1.16 |
| | E Med | 0.159 | 0.24 | | |
| | E Med (no Adriatic) | 0.147 | 0.19 | | |
| **April 1998** | W Med | 0.250 | 0.24 | 1.49 | 1.60 |
| | E Med | 0.168 | 0.23 | | |
| | E Med (no Adriatic) | 0.156 | 0.20 | | |
| **May 1998** | W Med | 0.212 | 0.19 | 1.29 | 1.40 |
| | E Med | 0.164 | 0.23 | | |
| | E Med (no Adriatic) | 0.151 | 0.17 | | |

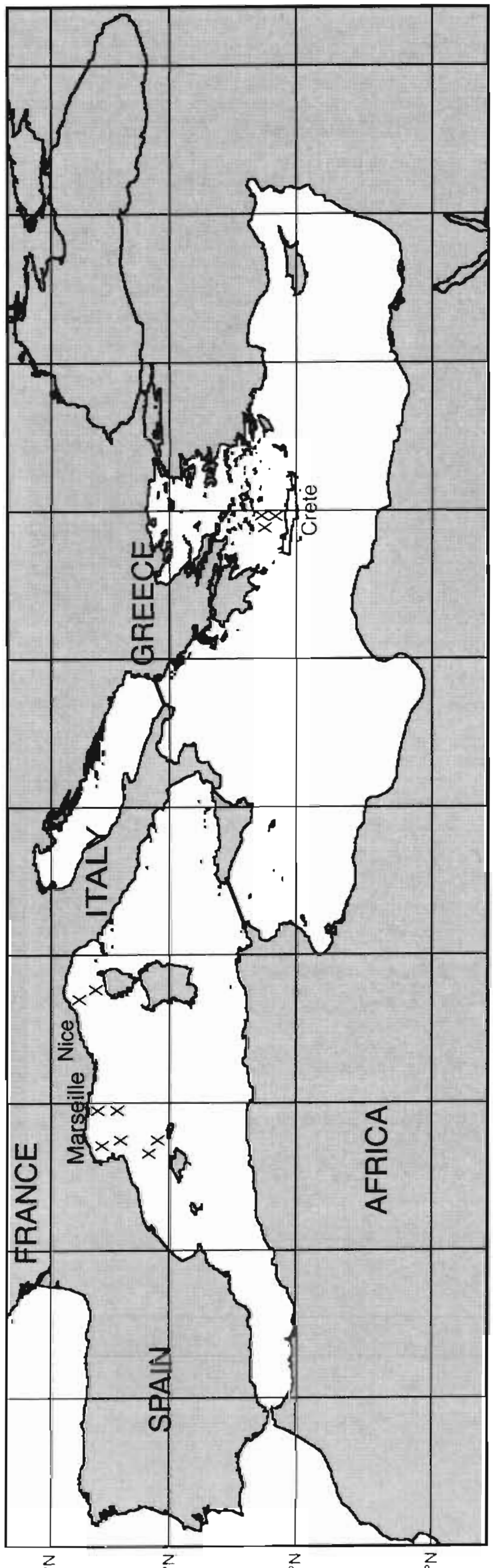


Fig. 2. Map of the Mediterranean Sea showing sampling stations and the lines used to demark western and eastern basins for the estimates of mean surface chlorophyll concentration for each basin (see Table 1 legend for details). (X) Station locations on the 3 cruises in the western (November 1994, April/May and July 1995) and 2 cruises in the eastern (Cretan Sea) (March and September 1995) Mediterranean

is over 3 times that in the east (Table 2). In other words, the phytoplankton efficiency in the eastern Mediterranean is a third of that in the west (Table 2). The phytoplankton efficiency may be an indication of the degree of nutrient and light limitation.

Similarly, the biomass of bacteria integrated to the base of the DCM (DCMb) is only slightly higher in the east than the west, but their growth rate is significantly lower in the east than in the west (Table 2). Hence, measures of biomass (chlorophyll *a* and bacterial counts) are similar in the east and west, but activities (production and growth rates) are different.

Organic matter flux into bacteria is one of the major pathways of material and energy flow in pelagic foodwebs (Azam et al. 1983, Cole et al. 1988, Azam & Smith 1991, Azam et al. 1992, Ducklow & Carlson 1992). Dissolved organic carbon (DOC) generated from primary production by a variety of means is taken up by bacteria and used for their growth and metabolism (Azam & Smith 1991, Azam et al. 1992, Ducklow & Carlson 1992). The proportion of primary production supporting bacterial production in marine environments is reported to vary from 10% to over 100% with a mean of 30 to 40% (Cole et al. 1988, Ducklow & Carlson 1992). In the western Mediterranean, bacterial production integrated to the DCMb comprises 9 to 46% (mean 21%) of the integrated primary production (Table 2). Assuming a bacterial growth efficiency of 20% (del Georgio et al. 1997 calculated a global median value of 24%), then 44 to 228% (mean 110%) of primary production may be routed through the DOC reservoir and support the bacterial carbon demand (BCD). Significantly, when primary production is low, BCD may therefore exceed primary production in the western Mediterranean (Fig. 3). Similar calculations for the east reveal integrated bacterial production of 18 to 54% (mean 34%) of the integrated primary production (Table 2) and suggest that at a bacterial growth efficiency (BGE) of 20%, around 89 to 268% (mean 170%) of primary production is required to support the BCD. Hence, net heterotrophy may be observed in both east and west during certain times of the year. The calculations suggest that either a greater proportion of the primary production may flow to the microbial food web in the eastern Mediterranean than in the western Mediterranean despite lower rates of bacterial production or that BGE is generally lower in the east than the west. These estimates are conservative as BGEs in oligotrophic waters may be lower (Kirchman et al. 1991, Carlson & Ducklow 1996).

The direct proportionality of bacterial production and primary production in the eastern Mediterranean, demonstrated in Fig. 3, suggests that bacte-

Table 2. Concentrations and rates of measurements integrated from the sea surface to the base of the deep chlorophyll maximum (DCMb) in the western and eastern Mediterranean Sea. The depths of the DCMb are also given. *T*-tests were carried out to indicate the significance of the difference between western and eastern data using logged data where appropriate. Original data sets are available in the EMPS (Bianchi et al. 1997) and CINCS (Tselepides et al. 1997) Mediterranean Targeted Project Final Reports

| Variable (unit) | | Western | Eastern | West:East | *t* | p |
|---|---|---|---|---|---|---|
| Depth of DCMb[a] | Range | 60–110 | 100–150 | | | |
| (m) | Mean | 79.0 | 113.3 | –34.3 | –3.74 | 0.002 |
| | SD | 15.2 | 21.6 | | | |
| | n | 10 | 6 | | | |
| Bacterial biomass | Range | 653–1589 | 1042–1828 | | | |
| (mg C $m^{-2}$) | Mean | 1026 | 1372 | 0.75 | –2.41 | 0.029 |
| | SD | 314 | 274 | | | |
| | n | 10 | 7 | | | |
| Bacterial production | Range | 26.5–191.6 | 8.0–130.6 | | | |
| (mg C $m^{-2}$ $d^{-1}$) | Mean | 90.4 | 48.5 | 1.87 | 1.93 | 0.072 |
| | SD | 54.2 | 39.2 | | | |
| | n | 10 | 7 | | | |
| Bacterial growth rate | Range | 0.040–0.130 | 0.006–0.086 | | | |
| ($d^{-1}$) | Mean | 0.080 | 0.035 | 2.27 | 3.15 | 0.007 |
| | SD | 0.035 | 0.026 | | | |
| | n | 10 | 7 | | | |
| Primary production | Range | 144.0–1143.1 | 39.3–243.4 | | | |
| (mg C $m^{-2}$ $d^{-1}$) | Mean | 502.7 | 151.0 | 3.33 | 2.74 | 0.018 |
| | SD | 342.2 | 91.6 | | | |
| | n | 10 | 4 | | | |
| Chlorophyll | Range | 5.6–58.7 | 15.0–64.3 | | | |
| (mg $m^{-2}$) | Mean | 29.2 | 25.7 | 1.13 | 0.16 | 0.875 |
| | SD | 19.46 | 19.3 | | | |
| | n | 10 | 6 | | | |
| Phytoplankton efficiency | Range | 0.66–2.96 | 0.21–1.12 | | | |
| (mg C mg $chl^{-1}$ $h^{-1}$) | Mean | 1.75 | 0.58 | 1.17 | 2.42 | 0.032 |
| | SD | 0.92 | 0.41 | | | |
| | n | 10 | 4 | | | |

[a]In the case of 1 eastern station, in the absence of chlorophyll, the depth of the DCMb was taken to correspond with that of the nearest comparable station

Table 3. Regression analysis of log bacterial production on log primary production relationships shown in Fig. 3. In each case the top line gives the variance due to the regression, whose significance is tested by the *F* ratio to the variance within regions. The second line gives the additional variance accounted for by using separate regressions for east and west. The significance is obtained here by looking at the ratio between this and the additional amount accounted for by using a separate regression for each site within either region (within region variance). Finally, the significance of the variation between hauls (vertical sections) at the same site (within region variance) is tested by taking the ratio of this to the pooled error variance for individual regressions on each haul (within haul variance). This variation within regions is always significant, i.e. all the relations are different for each haul you take. Compared with this variation between hauls, the relation of bacterial production to primary production is highly significantly different between east and west

| Source of variation | df | SS | Variance | *F* | p |
|---|---|---|---|---|---|
| Regression | 1 | 13.21278 | 13.21278 | 90.35963 | <0.001 |
| Between regions | 2 | 1.757998 | 0.878999 | 6.011301 | <0.01 |
| Within regions | 24 | 3.509385 | 0.146224 | 2.460079 | <0.005 |
| Within hauls | 51 | 3.031384 | 0.059439 | | |

rial production is entirely dependent on primary production products. The regression line in Fig. 3 for the east shows a constant proportionality between bacterial and primary production of 0.22, which, if all primary products are bacterially respired, is equivalent to a geometric mean BGE of 22% (95% confidence limits are 17 and 29%). This novel method of estimating bacterial growth efficiencies gives a BGE value similar to that used in the above calculations and by del Giorgio et al. (1997) and supports the recent measurements of lower estimates (Carlson & Ducklow 1996, del Giorgio et al. 1997). Bacteria therefore play a major role in organic carbon flow in both the east and west, but this

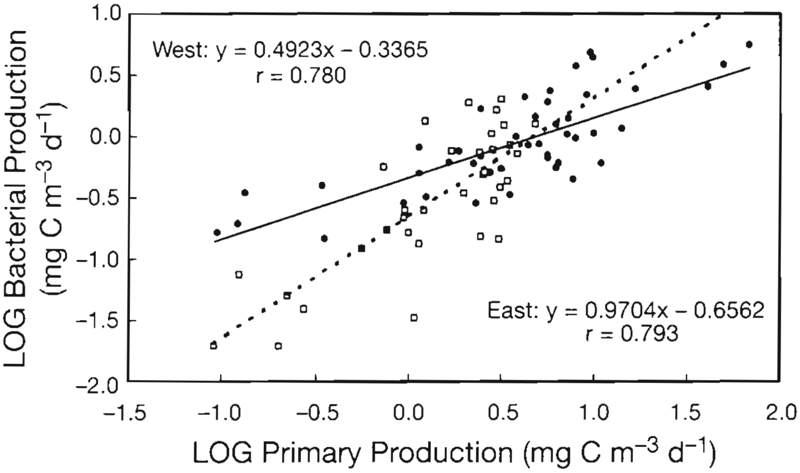


Fig. 3. The relation of log bacterial production to log primary production above the base of the deep chlorophyll maximum. The difference between west and east is significant ($F_{2,24} = 6.01$, $p < 0.01$, Table 3). Regression equations, statistics and lines are given for western (•; ——) and eastern (□; ----) Mediterranean data collected at different times of year (see Fig. 2 legend). Also see Table 3 for further statistical analysis

role is greater in the east, where microheterotrophs totally dominate the food web. In addition, small phototrophs dominate photosynthesis in oligotrophic waters favouring the dominance of a microbial loop, acting as an energy sink in the foodweb (Hagström et al. 1988).

The highly significant positive relationships between log bacterial production and log primary production (Fig. 3) for both the west and east Mediterranean indicate that primary production is a significant source of DOC for bacterial production in both areas. However, the relationship between bacterial production and primary production in the east and west are significantly different (Table 3). While bacterial production is directly proportional to primary production in the east, in the west it increases as approximately the square root of primary production (Fig. 3). Integrated primary production in the west is over 3 times higher than in the east (Table 2) and a DOC and DON reservoir is known to accumulate in the west during the summer (Copin-Montégut & Avril 1993, Pujo-Pay et al. 1997). The relation observed in Fig. 3 is consistent with periods or areas of high primary production effectively subsidising bacterial production in periods or areas of low primary production. In the west, when primary production is low, bacterial production is higher than it is in the east. If we assume the ecological efficiency of the conversion from photosynthetic products to bacteria to be similar in the east and west and that bacteria in the east are using essentially all the current primary production, this suggests that, when primary production in the west is low, bacteria are using some additional source of carbon. Conversely, when primary production is high, bacterial production in the west tends to be lower than would be predicted fromthe relation found in the east. It is notable that the maximum primary production in the east roughly corresponds with the point of intersection of the 2 lines.

The explanation we would suggest for these observations is that, when primary production is high (exceeding about 0.8 mg C $m^{-3}$ $d^{-1}$), the ecological efficiency of its bacterial utilisation declines and the excess organic carbon remains to be utilised when or where primary production is low.

This decline in efficiency may occur because bacterial populations may be unable to respond immediately to the production of DOC, due, perhaps, to nutrient limitation or predation resulting in DOC accumulation (Rivkin & Anderson 1997, Thingstad et al. 1997). In contrast, the gradient of close to 1 in the log bacterial production versus log primary production relationship in the east (Fig. 3) suggests very little spatio-temporal decoupling of this kind and, hence, little seasonal accumulation of DOC.

One explanation for the tight coupling between primary and secondary production in the east, may be exudation of carbon as mucopolysaccarides by nutrient stressed phytoplankton. Under extreme P-deficiency, as occurs particularly in the eastern Mediterranean (Krom et al. 1991), such production can be the main photosynthetic activity (Myklestad 1977). This may provide a better substrate for bacteria than for larger organisms (Azam & Smith 1991). Indeed, bacterial nutrient regeneration coupled with phytoplankton production of cell surface polysaccharides on this micrometer scale has been proposed as a self-sustaining mutualism between bacteria and phytoplankton especially in oligotrophic waters (Azam & Smith 1991). In contrast, the higher surface pigment concentration off southern France and Spain (Fig. 1a–c), due to high nutrient input from the Rhone, other rivers and local upwelling (Minas & Minas 1989), may act as a further source of DOC for bacteria in the western Mediterranean. In addition, the decoupling in the west may be exacerbated by high dispersion rates, seasonal vertical mixing, high settling rates, the production of relatively refractory DOC and changes in BGE.

Despite the strong coupling between individual measurements of bacterial production and primary production, the average integrated measurements of bacterial and primary production (Fig. 3) for the eastern Mediterranean fall on the general relationship described for a range of freshwater, estuarine and marine habitats (del Giorgio et al. 1997), where there is net heterotrophy during oligotrophy. In contrast, the western Mediterranean appears to support a biological system in which primary production and bacterial production are on average more balanced, despite being decoupled in space/time. As del Giorgio et al. (1997) suggest, some caution is required in such generalisations as the averaged integrated data used in their analysis masks the small spatial and temporal variability as seen in our high frequency data (Fig. 3) (see also Williams 1998). In the terms used by del Giorgio et al. (1997), the region we have sampled in the western Mediterranean may be regarded as in balance or as a net sink of $CO_2$, whereas the eastern Mediterranean may be regarded as a net source, reflecting the net autotrophic and heterotrophic oceanic provinces mentioned by Duarte & Agusti (1998), respectively.

Given that the bacteria above the DCMb in the east are utilising such a high proportion of the primary production, it is not surprising that there is little material remaining for the higher trophic levels and that there is a substantial west-east decrease in vertical mass flux which has also been linked to the increasing degree of oligotrophy (Heussner & Monaco 1996, Bianchi et al. 1996). Therefore, the degree of coupling between bacterial and primary production, in combination with the west-east decrease in primary production and the size of primary producers, may determine the lower pelagic and demersal fisheries (W:E ratio in fish production of 2.7:1) (Caddy & Oliver 1996), the lower vertical particle flux (W:E ratio about 9:1) (Heussner & Monaco 1996) and consequently the lower benthic biomass (W:E ratio in benthic biomass between 200 and 1000 m of 46:1) (Sara 1983) in the east.

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